REMARKS

Reconsideration is respectfully requested in view of the foregoing amendments, the following remarks, and the enclosed Rule 132 Declaration.

Claims 25, 34, and 39-44 have all been amended to recite "a single layer matrix". This is fully supported in the as-filed specification.

In the enclosed Declaration under 37 C.F.R. §1.132, the following experiments are described:

a) EXPERIMENT 1 + FIGURE 1: Experiment 1 substantially corresponds to the experiment described in Example 1 of the as-filed application, and Figure 1 in the Declaration corresponds to Figure 6 of the present specification. The results of this experiment show that when intestinal cells are seeded onto the hyaluronic acid ester matrix of the claimed invention, either in the form of a perforated membrane or in the form of non-woven tissue, they are able to grow better than on polyurethane, a material largely used for biomedical purposes, and used in this experiment as negative control. This is confirmed by the graph in Figure 1, that shows the activity of alkaline phosphates (ALP) in the cells grown on the various scaffolds tested. The higher the ALP, the greater the biochemical differentiation, and in this case ALP increases when going from polyurethane to the claimed non-woven matrix, and reaches its greatest value for the claimed matrix in the form of perforated membrane.

b) EXPERIMENT 2A) and 2B) + FIGURE 2: This experiment makes reference to two Examples of Dorigatti et al. (WO 94/17837) for the preparation of two different multilayer materials, which are then used as scaffolds for growing intestinal cells. The cell line and the operative conditions were the same as in Experiment 1. The results were completely unsatisfactory in both cases A) and B) and, as can be observed in Figure

2, the ALP activity measured as in experiment 1, was lower than that of cells grown on polyurethane, used as negative control in this experiment.

The Examiner states at page 2, item 2 of the Office Action, in the last paragraph, that Dorigatti et al. provide "*the general teachings that cells can be grown on nonwoven material comprising hyaluronic acid esters and/or a perforated membrane*".

The Examiner then notes that Dorigatti et al. "*is deficient in the sense, that the publication does not specify the type of cells, which may be grown on the material of the invention*".

Nevertheless, according to the Examiner (see last paragraph on page 3 of the Office Action), "*Valentini et al. provide hyaluronic acid matrices for the ingrowth of intestinal cells*".

Therefore, according to the Examiner, it would have been obvious for one of ordinary skill in the art to arrive at applicants' invention, i.e. growing intestinal cells on non-woven material, combining the teachings of Dorigatti et al. and of Valentini et al.

Applicants ask the Examiner to note that Dorigatti et al. refer to a multilayer material consisting of non-woven tissues layers only, or to a multilayer material consisting of non-woven tissues layers and a perforated membrane.

Moreover, Dorigatti et al. clearly state that the scope of their invention is to provide a biomaterial useful as skin coverage for repairing wounds (see for example Dorigatti et al., paragraph *Description of Related Art*, pages 1-2), and they do not disclose or suggest to use their multilayer materials for growing intestinal cells.

Now, Applicants have repeated the same experiment of the instant application, which is set forth in the attached Rule 132 Declaration, but using a multilayer material according to Dorigatti et al. as the scaffold, instead of the claimed single-layer material.

As is evident from the results in the enclosed Declaration, the multilayer material of Dorigatti et al. is *not* able to stimulate growth of intestinal cells, the ALP activity of the cells grown on it, being lower than that of cells grown on polyurethane (see Figure 2 of the Declaration).

Therefore, as to the Examiner's observation at page 5, item 6 of the Office Action that the test for obviousness is "*what the combined teachings of the references would have suggested to those of ordinary skill in the art (See In re Keller, 642 F.2nd 413, 208 USPQ 871 (CCPA 1981)*", the Examiner should take note that any ordinary skilled person aware of Dorigatti et al. and Valentini et al. would have tried to grow intestinal cells on the multilayer material disclosed by Dorigatti et al. but, as shown in the enclosed Declaration, these materials would have been entirely unsuitable to stimulate the growth of intestinal cells. Since one of ordinary skill in the art would have found the results to be unsatisfactory, the skilled person would not have been provided with any incentive to use the present single-layer matrices in the form of non-woven tissue or perforated membrane.

Besides, this would have been coherent with the teaching of Valentini et al. that a sponge matrix is suitable for growing intestinal cells, and the skilled person would have not found any further incentive in Valentini et al. to use the present matrices, which are completely different from and bear no similarity whatsoever to a sponge.

Finally, we would like to comment on the Examiner's observation at page 4, item 4 of the Office Action that "*the claims in the instant application do not read on a single layer material*".

Concerning this, we fully disagree with the Examiner and consider the claims prior to being amended herein which read "*a matrix chosen between a non woven fabric matrix and a perforated matrix*", to be as clear as possible on this aspect of the invention. It

would be evident to any skilled person reading those claims that they do not cover a multilayer material made of two or more matrices stuck together as those claimed by Dorigatti et al., but they cover a material made of a single matrix.

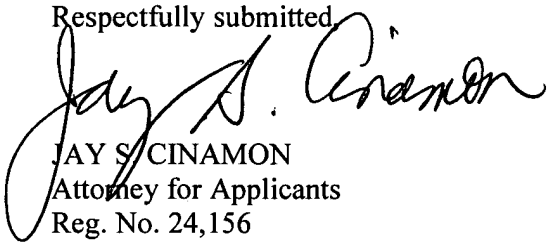
Notwithstanding the foregoing belief, applicants have amended independent claim 25 and all of the claims which depend (either directly or indirectly) from claim 25 to now recite "a single layer matrix".

In view of the foregoing amendments and the enclosed Rule 132 Declaration, the claims distinguish over the prior art. Accordingly, the §103(a) rejection has been overcome and should be withdrawn since a *prima facie* case of obviousness has not been established.

The issuance of a Notice of Allowance is respectfully solicited.

Please charge any fees which may be due, and which have not been submitted herewith, to our Deposit Account No. 01-0035.

Respectfully submitted,



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PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of

Applicant : CALLEGARO Lanfranco et al.
Serial No. : 09/743,333
Filed : February 21, 2001
Title : BIOCOMPATIBLE AND BIODEGRADABLE COMPOSITIONS CONTAINING HYALURONIC ACID AND THE DERIVATIVES THEREOF FOR THE TREATMENT OF ULCERS IN THE DIGESTIVE APPARATUS
Examiner : Liliana Di Nola-Baron
Art Unit : 1615

DECLARATION UNDER CFR 1.132

I, Anna Zanellato, being duly sworn depose and say that:

1. I am an Italian citizen residing in Bovolenta (Padua), Italy.

2. I am familiar with the English language.

3. I further declare that:

A) Universities or Colleges attended:

I graduated in Biology in the academic year 1987 at the University of Padua, Italy.

B) Publications

I am author and co-author of 19 scientific papers on peer reviewed journals, and inventor of two patent applications.

C) Professional experiences and research activity

From 1987 to 1990 I had worked at the University Department of General Pathology as a researcher, where I had been involved in a study pertaining to smooth muscles cells cultures and in particular to the mechanism of atherosclerosis.

Since 1990 I have been working at FIDIA FARMACEUTICI S.p.a. as a researcher,
my research activity involved :

- the analysis of action mechanism of trophic factors,
- studies, utilising neuronal cultures to select new chemical molecules pharmacolgically active to prevent different types of neuronal pathologies,
- other studies concerning bovine, rabbit, human , articular chondrocytes cultures on biomaterials.

4. I further declare that the following Experiments 1 and 2 were carried out under my direct responsibility and supervision, in order to demonstrate the ability of the matrix consisting essentially of hyaluronic acid derivatives according to the invention in acting as a support for growth of intestinal cells. With the present matrix and intestinal cells optionally together with fibroblasts, mesenchimal cells, mature cells and/or epithelial cells, a biological material can be prepared, which is suitable for regenerating the walls or filling diverticula of injured digestive apparatus and can be implanted onto the lesion site by surgical methods

EXPERIMENT 1

Intestinal cells were seeded onto scaffolds made of the total benzyl ester of hyaluronic acid in the form of a perforated membrane and onto scaffolds made of the total benzyl ester of hyaluronic acid in the form of a non-woven fabric, in order to test their biocompatibility, and their morphological and biochemical responses were observed.

The cells belonged to the $CaCO_2$ cell line (derived from human colon carcinoma) that differentiates spontaneously into enterocytes typical of the mature intestinal epithelium.

The cells were used at passage 98. They were seeded at a density of about 9 $x10^3/cm^2$ in DMEM 4.5 g of glucose/L containing 20% FBS penicillin/streptomycin, fungizone and non-essential amino acids (1%) in a humidified atmosphere with 95% CO_2. The culture medium was changed every 48 hours. Other cells were seeded on Petri dishes and Transwell wells with polycarbonate membranes in the same culture conditions and served as controls.

Polyurethane (Chronoflex TM), a material for biomedical purposes, was used as negative control. On the 3^{rd}, 15^{th}, 20^{th} and 40^{th} days of culture, the cells were prepared for observation user scanning electron microscope (SEM) and for assessment of the total proteins and the activity of alkaline phosphates (ALP) according to the following methods:

SEM fixing in 2.5% glutaraldehyde in phosphate buffer (PBS) pH 7.4. Osmium tetroxide, 1% in PBS, dehydration in ethanol and increasing concentrations of up to 100% and dehydration with a Critical Point drier. The cells were then metalized with gold and observed by SEM.

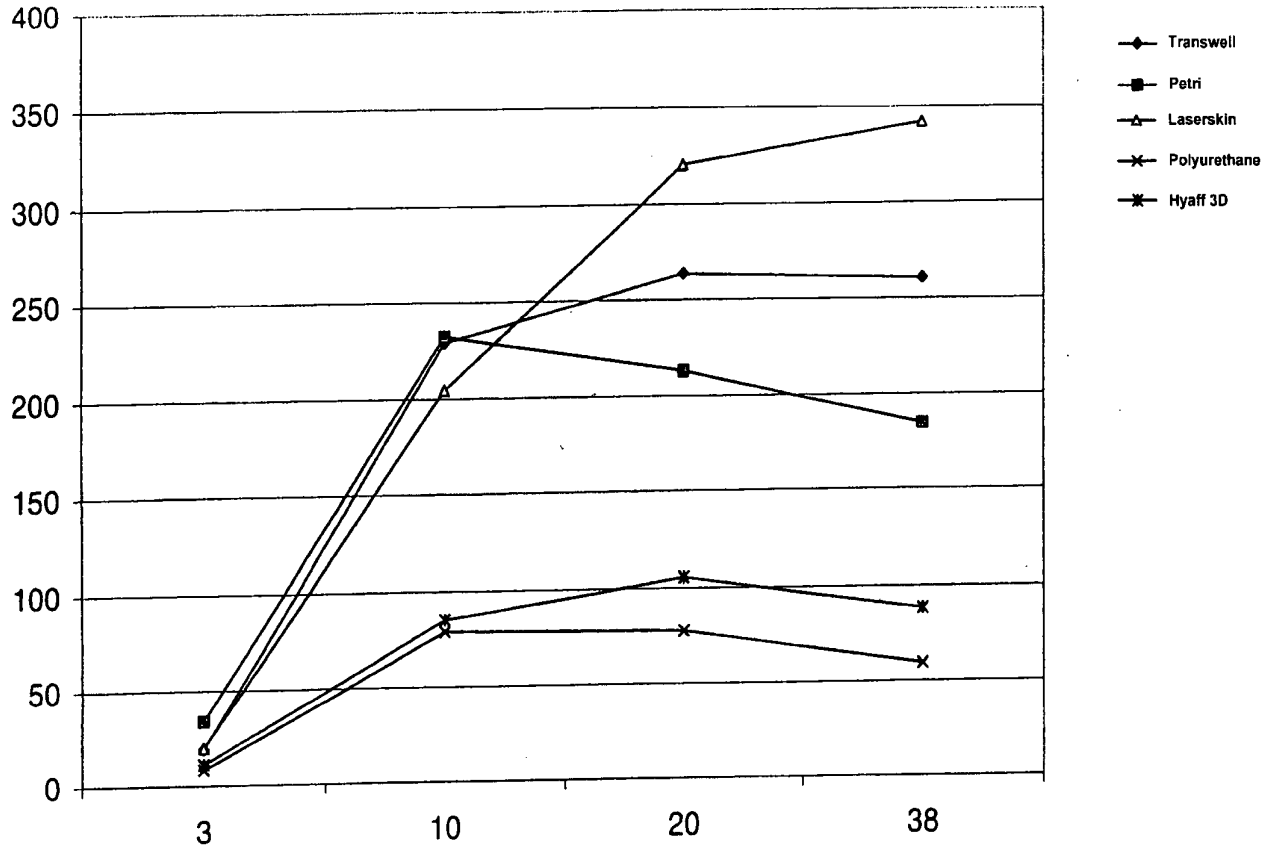
ALP activity: the cells were harvested by scraping in a lysis buffer 2mM Tris-HCl 50 mM mannitol pH 7.2 (1 ml final volume) (with the exception of those seeded on Hyaff 3D) and

sonicated in ice. ALP activity of the cellular lysates was determined by spectrophotometry by hydrolysis of the p-nitrophenylphosphate using a Boehringer kit. The total proteins were determined by Lowry's method. The activity present in the cells grown on a scaffold in the form of a non-woven fabric was determined in lysates obtained by sonicating the membrane containing the cells in toto. Morphological differentiation was assessed on the basis of the presence of microvilli on the upper surface of the cells, while the biochemical differentiation was assessed on the basis of the increase of ALP activity. Both were considered as biocompatibility parameters.

The results of ALP activity for cells grown on transwells, Petri dishes, total benzyl ester of hyaluronic acid in the form of a perforated membrane Laserskin®, total benzyl ester of hyaluronic acid in the form of non-woven tissue (Hyaff11 3D) and polyurethane, already obtained and showed in Figure 6 of the present application were confirmed by the present experiment and showed in the following Figure 1.

Electron microscope images were taken on the 38th day of culture of the cells grown on transwells, Petri dishes, total benzyl ester of hyaluronic acid in the form of a perforated membrane Laserskin®, total benzyl ester of hyaluronic acid in the form of non-woven tissue (Hyaff11 3D) and polyurethane membranes. These images showed practically the same results as in Figures 1, 2, 3, 4, 4a and 5 of the present application, and namely the cells grown on Laserskin and Transwell show marked differentiation due to the appearance of numerous microvilli on their surfaces, whereas those grown on Petri dishes show fewer, less well developed microvilli. The cells grown on the scaffold (in the form of a non-woven fabric) and Chronoflex do not show any formation of microvilli, while those grown on Chronoflex alone present extroversion indicative of cell suffering.

Figure 1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20
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Transwell
Petri
Laserskin
Polyurethane
Hyaff 3D

EXPERIMENT 2

In order to demonstrate that the matrices disclosed by Dorigatti et al. (WO 94/17837) would be unable to stimulate the growth of intestinal cells, the Experiment 1 was repeated with the same cell line and same conditions, but using the following two multilayer matrices chosen amongst those disclosed and prepared by Dorigatti et al.:

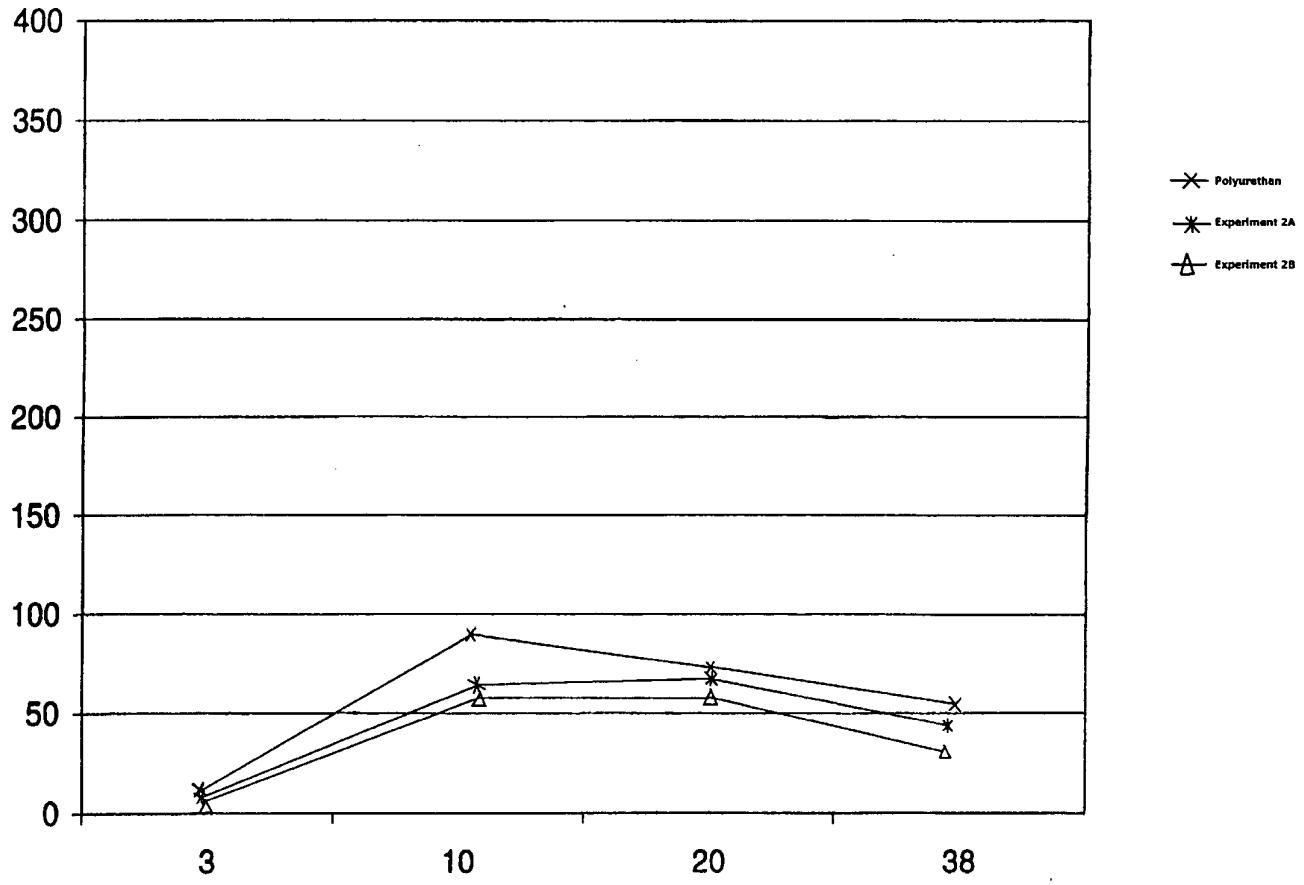
A) a multilayer non-woven tissue composed of a layer of non-woven hyaluronic acid benzyl ester, HYAFF 11, and a layer of non-woven viscose (Jettex 2005 from ORSA) with a basis weight of 80 g/m^2, a thickness of 2 mm, and water absorption percentage 560% by weight, prepared as disclosed in Example 1 of Dorigatti et al.

B) a multilayer non-woven tissue composed of a layer of non-woven hyaluronic acid benzyl ester, HYAFF 11, and a layer of non-woven viscose (Jettex 2005 from ORSA) with a basis weight of 80 g/m^2 and a thickness of 2 mm, joined to a HYAFF11 membrane perforated with 0.5 mm holes, prepared as disclosed in Example 7 of Dorigatti et al.

Cells belonging to the same cell line used in Experiment 1 were seeded onto the above two-layers matrices A) and B), on the side of non-woven layer of HYAFF 11.

In both cases A) and B) the cells revealed a slowed growth and did not show any formation of microvilli. The results of ALP activity are showed in Figure 2 for the two cases A) and B) and for a matrix of polyurethane used as negative control as in Experiment 1.

FIGURE 2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Polyurethan
Experiment 2A
Experiment 2B

5. I finally declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that such willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the applications or any patents or re-examination certificate issued thereon.

Abano Terme, (2004-07-09)

Anna Zanellato